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(54) **Title:** POTENTIATION OF ERYTHROPOIESIS

(57) **Abstract**

A method for modulating the rate of erythropoiesis in human hematopoietic progenitor cells. FRP is found to be an efficacious potentiator of, and inhibin a suppressor of, erythropoietin-induced differentiation. FRP and inhibin are shown to be functional antagonists of each other, and thus represent an effective means for modulating erythropoiesis in a number of disease states which are directly caused by or associated with an abnormal rate of erythropoiesis.

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POTENTIATION OF ERYTHROPOIESIS

The present invention relates generally to the field of hematology. More particularly, the invention relates to a method for augmenting or diminishing the rate of erythropoiesis in humans by administering an effective dose of FSH-releasing protein (hereinafter FRP) or inhibin, respectively.

Background of the Invention

Blood cells are derived from progenitor cells which are derived from hematopoietic stem cells. The process whereby it is determined whether a stem cell will ultimately differentiate to form erythrocytes or leukocytes is a complex and not well understood process thought to be controlled by hormonal signals received by the cells during maturation. Although the precise scheme of hematopoiesis is not presently understood, the role of some hematopoietically-active agents is.

The process by which stem cells are induced to become mature red blood cells, erythropoiesis, is under the control of erythropoietin, a glycoprotein hormone synthesized primarily in the kidneys. Erythropoietin, which itself is regulated by the number of circulating erythrocytes, induces progenitor cells to become committed to differentiate into erythrocytes.

Several mammalian cell lines serve as models for studying erythropoiesis. These cell culture models differ as to the species from which they were derived (e.g., mouse, human, etc.) and as to their relative stage of erythrodifferentiation. Both the species of origin and the stage of maturation of a cell line are important parameters which must be considered when studying erythropoiesis. Many biologically-active molecules involved in the process of red cell differentiation have a marked effect in one species, but no activity in another. Also, proteins which clearly affect erythrodifferentiation at one stage of

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development may have no effect at a later or earlier stage. Thus, narrow specificities in the activity of molecules active in erythrodifferentiation are commonly seen.

5   The K562 cell line is a human cell line characteristic of cells at a relatively mature stage in erythrodifferentiation. It expresses such red cell markers as glycophorin A, spectrin and antigen i. K562 cells undergo differentiation and accumulate hemoglobin

10   (one of several heme proteins, and the essential one for binding and transporting oxygen) upon induction by hemin or other chemical inducers.

A cell line that has been used to study erythrodifferentiation in mice is the mouse Friend

15   leukemia cell line. It is a model for differentiation established with Friend virus. Several chemical inducers that work in this mouse cell line do not work with human cell lines. Furthermore, the presently known chemical inducers for these cell lines are not the

20   physiological regulators for erythropoiesis in vivo.

Therefore, results derived from studies conducted on certain cell lines do not assure that the same biological agent will work in an in vivo system or in cells which may be at a different stage of

25   development and/or which may be derived from a different species.

In this respect, bone marrow is a significant source of progenitor cells for the study of erythrodifferentiation. Progenitor cells cultured from

30   bone marrow respond to many physiological hematopoietic factors found in vivo.

A preparation of placental extract was shown by Ikawa et al., Gann. 66, 583-584 (1975) to enhance δ-amino-levulinic acid synthetase and the percentage of benzidine

35   staining cells (i.e., heme-containing cells) in a mouse Friend leukemia cell culture. Recently, using the same

mouse cell line, Eto, et al., _Biochem. Biophys. Res._
Comm. 142, 1095-1103 (1987), reported that EDF
(erythroid differentiating factor) increased the
percentage of cells staining for an unidentified heme

5   protein, and that EDF and porcine FRP had similar
amino-termini.

Inhibin, a heterodimeric protein consisting of
an α -subunit (18,000 daltons) and one of two ß
subunits (β_A or β_B) (14,000 daltons), and FRP, a

10   homodimer consisting of a pair of ß subunits, are
functionally antagonistic, hypophysiotropic hormones
which were shown by Vale, et al., _Nature_ 321, 776-779
(1986) to respectively suppress and enhance the
secretion of follicle-stimulating hormone (FSH).

15   We have found, unexpectedly, that FRP and
inhibin exert a novel and complex humoral regulatory
control over erythropoiesis in humans. FRP induces
hemoglobin accumulation in K562 cells, and inhibin
functionally antagonizes this effect. In addition to

20   the regulatory effect on hemoglobin accumulation, FRP
and inhibin have profound augmenting (FRP) and
suppressing (inhibin) activity on erythropoietin-induced
proliferation and differentiation of bone marrow
progenitor cells.

25   Summary of the Invention
One method of the invention relates to
increasing the number of red blood cells (RBCs) in the
bloodstream and/or the hemoglobin concentration in
blood. FRP potentiates the proliferation and

30   erythrodifferentiation of erythroid progenitor cells
cultured from bone marrow, and the differentiation and
accumulation of hemoglobin in K562 cells. Although FRP
potentiation of bone marrow cultures is dependent on the
presence of erythropoietin to initiate erythrodifferenti-

35   ation, FRP is a very efficacious enhancer of
erythropoiesis in both cell culture systems.

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K562 cells treated with picomolar concentrations of FRP accumulate hemoglobin and become terminally differentiated with limited capacity for growth. The increase in the concentration of hemoglobin in erythroid cells on a single cell basis, when compared to other inducers of erythroid differentiation, is about double (i.e., although certain compounds may induce more cells to differentiate than does FRP, cells in which erythrodifferentiation was potentiated by FRP produce about twice the amount of hemoglobin on a per cell basis).

FRP similarly causes up to a 300% increase in erythrodifferentiation in bone marrow cultures induced with erythropoietin, as compared to inducement with erythropoietin alone.

Inhibin is the functional antagonist of FRP, suppressing spontaneous erythrodifferentiation in K562 cell cultures, FRP-induced erythrodifferentiation in K562 cells, and FRP-potentiated differentiation in bone marrow cultures induced with erythropoietin. FRP and inhibin thus provide a novel humoral regulatory effect on erythrodifferentiation in much the same manner as inhibin and FRP have been shown by Vale, et al. supra, to regulate gonadotropin homeostasis.

Detailed Description of the Preferred Embodiment

The term "CFU-E" or "erythroid colony-forming unit" as used herein refers to a progenitor cell derived from an hematopoietic stem cell which, when induced by erythropoietin, becomes committed to proliferate and differentiate to generate a colony of about 15-60 mature erythrocytes (which can be recognized in 7 days in a human bone marrow culture).

As used herein the term "potentiate" refers to the ability of FRP to augment the rate of commitment to erythroid differentiation.

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The term "erythrodifferentiation-potentiating capacity" as used herein refers to the relative difference in the number of erythropoietin-inducible CFU-Es with and without a maximally-effective amount of FRP.

One embodiment of the present invention is directed to a method for in vivo regulation of the number of progenitor blood cells that will differentiate into erythroid cells and regulating the concentration of hemoglobin in blood. The method is useful for increasing hemoglobin in treating diseases like anemia or hypoxia in which symptoms are caused by abnormal or otherwise undesirable hemoglobin or red blood cell count.

Augmenting the rate of erythropoiesis with FRP is clinically useful for treatment of congenital or acquired aplastic or hypoplastic anemia; amelioration of anemia associated with cancer, AIDS, chemotherapy, radiotherapy, bone marrow transplantation and chronic diseases; increasing red cell production in long distance runners and patients undergoing elective surgery; countering hypoxia at high altitude. Lowering the rate of erythropoiesis with inhibin is useful in the treatment of polycythemia vera and erythroleukemia. Furthermore, FRP and inhibin may control the growth and differentiation of erythroleukemia and other malignant cells.

In some clinical situations, modulating the rate of erythrodifferentiation may be useful to lower the rate of red cell production. For example, to afford a measure of protection to erythropoietic precursor cells from chemotherapeutic agents, the rate of erythropoiesis may be lowered by administration of inhibin prior to therapy, thus putting erythroid precursors in a quiescent state. Thereafter, FRP may be given to overcome the suppressing effect of inhibin and reestablish a normal rate of erythropoiesis.

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FRP and inhibin may be administered, by
injection, ingestion, inhalation, or other methods, such
as intranasal administration. It is preferred that the
method of administration be parenteral, such as by
intravenous, subcutaneous or intramuscular injection or
otherwise. The daily dosage of FRP or inhibin should be
between about 1-50 μg of protein/kilogram of body
weight, provided that the preparation is substantially
pure (~98-100%) and of high specific activity. The
dimers may be in the form of pharmaceutically acceptable
non-toxic salts, such as acid addition salts or metal
complexes, e.g., with zinc, iron, calcium, barium,
magnesium, aluminum or the like. Illustrative of such
acid addition salts are hydrochloride, hydrobromide,
sulphate, phosphate, tannate, oxalate, fumarate,
gluconate, alginate, maleate, acetate, citrate,
benzoate, succinate, ascorbate, tartrate and the like.
If the active ingredient is to be administered in tablet
or like form, the tablet may contain a pharmaceutically
acceptable binder or carrier, such as tragacanth, corn
starch or gelatin; a disintegrating agent, such as
alginic acid; and a lubricant, such as magnesium
stearate. If administration in liquid form is desired,
sweetening and/or flavoring may be used, and intravenous
administration in isotonic saline, phosphate buffer
solutions or the like may be effected.

It is also contemplated that in vivo
erythropoiesis may be augmented in vitro by obtaining a
sample of bone marrow cells, as is known in the art,
potentiating erythropoietin-induced differentiation with
FRP and infusing the FRP-treated cells back into the
patient.

Another embodiment of the invention concerns
assaying the erythropoietic capacity of an individual.
In certain disease states such as anemia and hypoxia,
erythropoietin levels may be elevated. It would be
desirable to know the capacity of the hematopoietic

system to produce still more red blood cells by potentiating erythropoiesis with FRP. An analysis of such a capacity for an increased rate of red cell generation would be useful to a physician determining a
5   course of treatment. Furthermore, even if erythropoietin levels are normal, FRP can potentiate whatever normal rate of erythropoiesis is present.

One assay for making such a determination utilizes freshly obtained bone marrow precursor cells or
10   precursor cells from another appropriate source which cells are seeded in culture vessels in a suitable growth medium containing methylcellulose for growth and detection of CFU-Es. Such cultured cells are induced with an effective amount of erythropoietin with or
15   without a potentiating amount of FRP. The cultures are incubated for a suitable time at 37°C. in a humidified atmosphere (95% air; 5% CO_2) to allow CFU-E formation and the number of colonies are scored and compared to determine the degree of potentiation that FRP caused.

20   Another assay can essentially be performed in vivo by administering FRP and monitoring erythropoiesis by red cell count or hemoglobin concentration with time.

A further embodiment of the invention encompasses a diagnostic kit. In addition to
25   erythropoietin and FRP, a kit may include means for obtaining hematopoietic precursor cells, suitable culture medium for carrying out growth of precursor cells through the stage of CFU-E formation, and tissue culture vessels suitable therefor. Because tissue
30   culture vessels, suitable growth medium, and other supplies and instruments may be commonly available in the laboratory, a diagnostic kit could consist of FRP and erythropoietin of suitable purity for assay purposes. Generally what is required for suitable
35   purity may be empirically determined as one skilled in the art would appreciate.

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K562 cells were cultured in RPMI 1640
supplemented with 15% fetal calf serum with and without
various concentration of FRP. Incubation of the cells
with FRP for 3 days did not adversely effect K562 cell
proliferation, as compared to control cell cultures. A
clonal assay (see Rowley, et al., Blood 65, 862-868
(1985), in which cells were grown in RPMI 1640 culture
medium containing 0.8% methylcellulose supplemented with
0, 1, 5 or 10 ng/ml FRP showed that plating efficiencies
were 97% (1 ng/ml), 90% (5 ng/ml) and 92% (10 ng/ml).
Thus, FRP is not cytotoxic to K562, as are some chemical
inducers known in the art.

FRP induces an increase in the rate of
erythroid differentiation in K562 cells. Clonal assays
performed on K562 cells in the presence of FRP revealed
that colonies grown in the presence of FRP were smaller
(about 16 cells) than colonies formed in the absence of
FRP (predominantly 32+ cells). That the FRP treated
cultures, but not the control cultures, had limited
capacity for further growth, and that the cultures
stained benzidine-positive for hemoglobin was indicative
of being erythrodifferentiated. Thus, picomolar
concentrations of FRP induce K562 cells to become
terminally differentiated.

K562 cells grown in RPMI 1640 with 15% fetal
calf serum spontaneously differentiated at a relative
low rate (i.e., approximately 6%). K562 cells grown in
the same medium except supplemented with FRP show
significantly higher rate of erythrodifferentiation.
Culture medium made 300 picomolar with respect to FRP
(10 ng/ml) induced the maximum amount of cells which
stained benzidine-positive. Greater concentrations of
FRP failed to induce more cells to differentiate. The
half-maximal rate of induction occurred at approximately
1 ng/ml (i.e., 30 pM). Thus, the capacity to induce
differentiation at such low concentration indicates that
K562 cells have high affinity receptors for FRP.

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The amount of hemoglobin in K562 cells treated
with or without FRP or hemin was determined by cell
lysis and measurement of hemoglobin by the
spectrophotometric assay described in Tsiftsoglou, et
al., _Cancer Res._ 39, 3849-3855 (1979), and the results
are represented in Table I.

TABLE I

Hemoglobin Contents in Control
and Various Induced K562 Cells

Inducers	Hb Content/ 10^6 cells	Benzidine Staining Cells
1. None	0.06 μg	6%
2. FRP, 10 ng/ml	1.37 μg	48%
3. Hemin, 25 μM	1.50 μg	94%

Table 1 shows that 48% of the FRP induced cells
stained positive as compared to 94% of hemin induced
cells. Importantly, however, FRP-treated cultures
produced essentially as much hemoglobin per culture as
did the hemin-treated cultures. Thus, on a single cell
basis, FRP is approximately twice as effective in
inducing hemoglobin production as is hemin. FRP is
similarly efficacious when compared to other chemical
inducers of erythrodifferentiation (data not shown).
The hemoglobin isolated from lysed FRP-treated cells is
shown to be mainly embryonic as assessed by
immunoassays, globin chain analyses and isoelectric
focusing gels.

FRP, in picomolar concentrations, also
potentiates erythropoietin-induced differentiation of
human bone marrow progenitor cells. Bone marrow cells
grown in culture will erythrodifferentiate only if the

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culture medium is supplemented with erythropoietin.
Erythropoietin is known to cause certain progenitor
cells, that is CFU-Es, to proliferate (i.e., undergo a
series of divisions) which proliferation terminates with
the production of differentiated, mature red blood
cells. In Table II, below, inducement of erythroid
progenitor cells to differentiate was assessed by
scoring hemoglobinized colonies expressing after 7 days,
the well-known, unique morphology characteristic of
CFU-Es.

TABLE II

Effect of FRP on Colony
Formation of CFU-E from Human Bone Marrow

	Units/ml of Erythropoietin			
	0	0.2	0.5	3.0
Additive:	Mean Colony Number $\pm$ S.D./10^5 Cells			
1. None	0	13.3 ± 2.5 (100%)	26.3 ± 2.3 (100%)	41.3 ± 5.1 (100%)
2. FRP, 1 ng/ml	0	17.7 ± 2.5 (133%)	34.3 ± 7.3 (130%)	78.7 ± 8.5 (191%)
3. FRP, 5 ng/ml	0	24.0 ± 3.6 (181%)	39.7 ± 5.6 (151%)	94.3 ± 7.6 (228%)
4. FRP, 10 ng/ml	0	39.3 ± 3.5 (296%)	43.6 ± 6.5 (166%)	121.6 ± 17.8 (295%)

As shown by Table II, FRP has an enhancing
effect on erythropoietin-induced differentiation in bone
marrow cultures. Although 1 ng/ml of FRP might not have
had a statistically significant enhancing effect, the
addition of more than 5 ng/ml of FRP to cultures in the
presence of erythropoietin did significantly potentiate
proliferation of CFU-E colonies ($p < 0.01$ in the t-test).

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As compared to the control culture which received
erythropoietin alone (row 1), FRP caused approximately a
150-300% increase (rows 3 and 4).

Inhibin exerts a suppressive action which
opposes induction of erythroid differentiation,
especially when it is FRP-potentiated. This result is
analogous to the earlier findings that inhibin and FRP
exert opposite biological actions on hormone secretions
by pituitary cells which effectively modulate hormone
levels. Table III represents the results of an
experiment in which K562 cells were cultured with or
without inhibin and/or FRP for 3 days and then stained
with benzidine.

TABLE III

Effect of FRP and Inhibin on
Induction of K562 Cells to Differentiation

	FRP (ng/ml)			
	0	1	5	10
	Benzidine Staining Cells (%)			
Inhibin, 0 ng/ml	6 ± 1	23 ± 3	32 ± 6	41 ± 3
Inhibin, 1 ng/ml	1 ± 1	8 ± 2	21 ± 2	26 ± 5
Inhibin, 5 ng/ml	3 ± 2	8 ± 1	12 ± 3	16 ± 2
Inhibin, 10 ng/ml	3 ± 1	3 ± 2	9 ± 1	11 ± 2
Inhibin, 50 ng/ml	2 ± 1	4 ± 1	6 ± 4	9 ± 1

As shown, the addition of inhibin to cultures
suppressed the FRP-induced differentiation in a
dose-responsive manner. The level of half-maximal
suppression was in the range of 1-5 ng/ml. The data
suggest that inhibin alone did not induce
differentiation, and seems to in fact suppress
spontaneous differentiation.

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Inhibin exhibited an analogous suppressing activity on inducement of differentiation in bone marrow cells cultures (see Table IV). Bone marrow cells were cultured using, with slight modification, the methylcellulose technique described by Ogawa, et al., Blood, 48, 407-417 (1976). Briefly, 1-2 X10^5 mononuclear cells were plated in 35 mm petri dishes in 1 ml of medium, 30% fetal calf serum, 0.1 mM -thioglycerol, 50 I.U./ml penicillin and 50 ug/ml streptomycin. FRP and/or inhibin were added in accordance with the values stated in Table IV. The dishes were incubated at 37C in a humidified incubator flushed with 5% CO_2. Incubation was carried out for 7 days and then dishes were examined on an inverted microscope for hemoglobinized colonies of CFU-E. Data on the effects of erythropoietin and FRP were subjected to two-way ANOVA statistical analysis as described at pages 321-371 in Sokol, et al., Biometry (W. H. Freeman and Company, 1981). The critical values for these two analyses of treatments are $F_{[3,32]}=370.4$, $F_{[3,32]}=49.5$, respectively. Similar analyses were employed to analyze the effect of inhibin by comparing the data obtained from cultures which contained erythropoietin and similar amounts of FRP with or without excess inhibin. Numbers within parentheses represent the percentage of CFU-E colony formation relative to control cultures containing similar doses of erythropoietin, but no other additives (i.e., sample #1). The symbol, n.d., represents "no determination".

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TABLE IV

Effect of FRP and Inhibin on Colony Formation of CFU-E From Human Bone Marrow

		Units/ml of Erythropoietin			
		0	0.2	0.5	3.0
Additives:		Mean Colony Number $\pm$ S.D./10^5 Cells			
1.	FRP, none	0	13.3$\pm$2.5 (100%)	26.3$\pm$2.3 (100%)	41.3$\pm$5.1 (100%)
2.	FRP, 1 ng/ml	0	17.7$\pm$2.5 (133%)	34.3$\pm$7.3 (130%)	78.7$\pm$8.5 (191%)
3.	FRP, 5 ng/ml	0	24.0$\pm$3.6 (181%)	39.7$\pm$5.6 (151%)	94.3$\pm$7.6 (228%)
4.	FRP, 10 ng/ml	0	39.3$\pm$3.5 (296%)	43.6$\pm$6.5 (166%)	121.6$\pm$17.8 (295%)
5.	FRP none + Inhibin, 10 ng/ml	0	6.3$\pm$2.9 (47%)	18.3$\pm$3.0 (70%)	40.6$\pm$11.0 (98%)
6.	FRP, 1 ng/ml + Inhibin, 5 ng/ml	0	12.3$\pm$4.0 (93%)	19.0$\pm$3.5 (72%)	54.7$\pm$5.5 (133%)
7.	FRP, 5 ng/ml + Inhibin, 10 ng/ml	0	9.3$\pm$2.1 (70%)	19.3$\pm$4.0 (73%)	46.7$\pm$3.2 (113%)
8.	FRP, 10 ng/ml + Inhibin, 25 ng/ml	0	n.d.	n.d.	38.3$\pm$6.4 (93%)

As the table indicates, inhibin suppressed erythro-poietin-induced CFU-E formation, even when potentiated by FRP. This inhibitory effect was strongest at lower concentrations of erythropoietin; the suppressing effect of inhibin was not present at the highest concentration of erythropoietin (i.e., 3.0 units/ml). At 1 ng/ml of FRP, the addition of inhibin reduced the potentiating effect produced by FRP to about 70% of that expressed by

culture which had 1 ng/ml of FRP without inhibin; and at higher concentration of FRP, the suppressive effect of inhibin became more prominent. For example, in the culture containing 10 ng/ml of FRP and excess inhibin 32% of CFU-E were produced, compared to the number produced in the culture receiving 10 ng/ml FRP without inhibin.

The results of the experiment represented in Table IV indicate that FRP and inhibin are functionally antagonistic proteins which modulate the proliferation and differentiation of erythroid progenitor cells from bone marrow. As noted above, biological activity in picomolar concentrations is indicative of the progenitor cells having receptors for FRP and inhibin. These cells may have receptors with overlapping specificities or they may have distinct receptors specific for FRP and inhibin which are then functionally antagonistic, such that the binding of inhibin interferes with initiation erythropoiesis. Alternatively, the action of inhibin might be explained if FRP, which may be culture produced, mediated the suppressive effect of inhibin. The similarities between this discovery and earlier findings respective to observations of the effects of FRP and inhibin on FSH secretion in pituitary cells, point to a mechanism in which each of these two proteins has an independent basis for activity in the absence of the other. Therefore, regardless of the precise mechanism, the bone marrow culture experiments indicate that FRP and inhibin provide humoral regulation over erythropoiesis.

FRP and inhibin has been isolated in substantially pure form from follicular fluid of various species, e.g., porcine, as well as from rete testis fluid. FRP may be purified from follicular fluid or rete testis fluid using the method of Vale, et al., Nature, 321, 776-779 (1986), the disclosure of which is

hereby incorporated by reference. Briefly, FRP is
purified using ammonium sulfate precipitation, several
RP-HPLC steps, cation exchange chromatography, and gel
permeation chromatography. A method of purifying
5   inhibin, employing ammonium sulfate precipitation,
several reverse phase-high pressure liquid
chromatography (RP-HPLC) steps, and gel permeation
chromatography, is described in Rivier, J., <u>Biochem.</u>
<u>Biophys. Res. Comm.</u>, <u>133</u>, 120-127 (1985), the disclosure
10   of which is hereby incorporated by reference.

        The complete amino acid sequences of the two
forms of both porcine inhibin and of human inhibin have
been shown by Mason, A., et al., <u>Nature</u>, <u>318</u>, 659-663
(1985) and <u>Biochem. Biophys. Res. Comm.</u>, <u>135</u>, 957-964
15   (1986), respectively, and the disclosures of these
articles are hereby incorporated by reference. Thus,
three distinct genes which code for the $\alpha-$, β_A-,
and β_B subunits are responsible for the synthesis of
such inhibin-related gene products.

20   Once a substantial portion of the sequence of a
protein hormone is known, the mRNA encoding the hormone
can be isolated. In this case, the mRNAs for both
chains are isolated and the cDNA's are synthesized by
recombinant DNA techniques. Messenger RNA (mRNA) is
25   obtained from ovarian follicles which produce inhibin,
and then cDNA is synthesized from the mRNA by reverse
transcription. The cDNA is inserted into a cloning
vector which is used to transform a suitable host to
create a cDNA library.

30   More specifically, based upon the known partial
amino acid residue sequence of the inhibin chains,
labeled oligonucleotides are synthesized for detecting
cDNA corresponding to each chain. Because of the
degeneracy of the genetic code, mixed hybridization
35   probes are prepared and used as probes. These probes
are then used to select, from the library, cDNA clones

that contain gene sequences encoding the chains. cDNA
libraries may also be screened by immunological
expression assay with an antibody raised against inhibin
or one of the two inhibin chains. Immunological
5   expression assay may also be used to confirm screening
with hybridization probes.

From selected clones, cDNA is excised and
inserted into appropriate vectors under the control of
suitable promoter sequences, and the vectors are
10   transformed into cell lines for expression of the
recombinant inhibin chains. Although vectors containing
the genes for both chains could conceivably be
transformed into the same cell line, for simplicity,
vectors for expression of each chain are preferably
15   transformed separately into cell lines. The two subunit
chains can then be isolated from the cellular material
and/or the cell culture medium. The two chains are then
subjected to oxidizing conditions which promote
disulfide bonding between the chains.

20   It can be appreciated that using this method,
various dimers of inhibin-related gene products will
likely be synthesized (e.g., $\alpha \beta_A$, $\alpha \beta_B$,
$\beta_A\beta_A$, $\beta_B\beta_B$, $\beta_A\beta_B$). Moreover, because it has
been shown that the ß-subunit is highly conserved in its
25   amino acid sequence, the possibility exists to
synthesize dimers wherein each subunit is derived from a
different species, which may provide certain advantages
over native dimers.

Moreover, European Patent Application S.N.
30   222 491 discloses a synthesis of FRP and inhibin by
recombinant DNA techniques, the disclosure of which is
incorporated herein by reference.

While the invention has been described with
specificity, modifications apparent to those with

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ordinary skill in the art may be made without departing
from the scope of the invention.

Various features of the invention are set forth
in the following claims.

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What Is Claimed Is:

1. As a new use for the modulation of erythropoiesis of human hematopoietic stem cells in the presence of an inducibly-effective concentration of erythropoietin a potentiating concentration of FRP or a suppressing concentration of inhibin.

2. As a new use according to Claim 1 the treatment of anemias resulting from an abnormality in the rate of erythropoiesis by a potentiating concentration of FRP in the presence of an inducibly-effective concentration of erythropoietin.

3. As a new use according to Claim 1, treatment of a disease from the group consisting of congenital aplastic anemia and acquired aplastic anemia, by a potentiating concentration of FRP in the presence of an inducibly-effective concentration of erythropoietin.

4. As a new use according to Claim 1, the treatment of an anemia which is associated with either cancer or AIDS, by a potentiating concentration of FRP in the presence of an inducibly-effective concentration of erythropoietin.

5. As a new use according to Claim 1, the treatment of an anemia associated with a chronic disease, bone marrow transplantation, radiotherapy or chemotherapy, by a potentiating concentration of FRP in the presence of an inducibly-effective concentration of erythropoietin.

6. As a new use according to Claim 1 the increase of red cell production in long distance runners or subjects of elective surgery, or to counter hypoxia at high altitude, by a potentiating concentration of FRP in the presence of an inducibly-effective concentration of erythropoietin.

7. As a new use according to Claim 1, the control of growth and differentiation of malignant cells

or the treatment of polycythemia vera or
erythroleukemia, by a potentiating concentration of FRP
in the presence of an inducibly-effective concentration
of erythropoietin.

8.   As a new use according to Claim 1, the
suppression of the rate of erythropoiesis prior to
chemotherapy or radiotherapy, a suppressing
concentration of inhibin.

9.   A composition for modulating erythropoiesis
of human hematopoietic stem cells comprising either an
effective potentiating concentration of FRP or an
effective suppressing concentration of inhibin, in
admixture with a pharmaceutically acceptable carrier.

10. A method for determining the
erythrodifferentiation-potentiating capacity of a human
comprising:

administering at least one effective dose of
FRP and determining the potentiating response by
monitoring red blood cell count or hemoglobin
concentration.

11. A method for determining the
erythrodifferentiation-potentiating capacity of
hematopoietic stem cells comprising:

administering an effective potentiating
concentration of FRP to a first representative portion
of hematopoietic stem cells in the presence of an
inducibly effective concentration of erythropoietin;

culturing said cells for a suitable length of
time in a culture medium suitable for forming and
detecting CFU-E; and

comparing the number of CFU-Es to a control
culture.

12. A diagnostic assay kit for use according
to Claim 11 to test the capacity of hematopoietic stem
cells to undergo erythrodifferentiation which kit
comprises an effective dosage of erythropoietin and FRP.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 88/04063

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC^4: A 61 K 37/43; A 61 K 37/24; // (A 61 K 37/43, 37:24)

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC^4	A 61 K

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	WO, A, 87/05810 (THE SALK INSTITUTE FOR BIOLOGICAL STUDIES) 8 October 1987	1-12
A	WO, A, 86/00078 (MONASH UNIVERSITY) 3 January 1986	1-12

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* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
6th March 1989	2 8 MAR 1989

International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	P.C.C. VAN DER PUTTEN

Form PCT/ISA/210 (second sheet) (January 1985)

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 17/03/89
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A- 8705810	08-10-87	AU-A- 7281887 EP-A- 0261239 JP-T- 63502989	20-10-87 30-03-88 02-11-88
WO-A- 8600078	03-01-86	AU-A- 4437485 EP-A- 0185034 JP-T- 61502399	10-01-86 25-06-86 23-10-86